REMARKS

Favorable reconsideration, reexamination, and allowance of the present patent application are respectfully requested in view of the foregoing amendments and the following remarks. The foregoing amendments are fully supported by the specification and original claims, at least on page 17 of the specification, in the 2nd and 3rd complete paragraphs, and page 12, the last paragraph. No new matter is added.

Amendments

Claims 1, 5, 13, 16, and 19 are amended. Claims 2, 3, 6, 7, and 17 are cancelled. Claims 8-11 are withdrawn. Claims 20-21 are new. Claims 1, 5, 13, 16, 19, and 20-21 are under examination.

Objection to the Claims

At page 2 of the Office Action, Claims 1, 5, and 16 were objected to because they allegedly are indefinite. Applicant respectfully requests reconsideration of this objection.

Claims 1, 5, and 16 have been amended to delete the term "sequence" as suggested by the Examiner to overcome this rejection.

For at least the foregoing reasons, Applicant respectfully submits that the Claims are not objectionable, and therefore respectfully requests withdrawal of the objection thereto.

Rejection under 35 U.S.C. § 112, first paragraph

In the Office Action, beginning at page 3, Claims 1, 4-5, 7, and 12-19 were rejected under 35 U.S.C. § 112, first paragraph, as reciting subject matters that allegedly fail to comply with the written description and scope of enablement requirements. Applicant respectfully requests reconsideration of this rejection.

Claims 1, 4-5, 7, and 12-19 are rejected as failing to comply with the written description requirement and the enablement requirement.

Regarding claim 1, this claim has been amended to recite that the homology level of the recited DNA with SEQ ID NO: 1 is "95% or more". This amendment is supported by the description on page 12, lines 16-19 of the present specification.

Regarding claims 5 and 16, these claims have been amended to recite that the homology level of the recited DNA with SEQ ID NO: 3 is "95% or more". This amendment is supported by the description on page 15, line 20 to page 16, line 5 of the present specification. Also, claims 5 and 16 have been amended to recite that the expression of glutamine synthetase gene is increased by increasing the copy number of the gene, or by replacing promoter region of the gene with a stronger promoter. This amendment is supported by the subject matter of claims 7 and 17, and the description on page 17, lines 15-21 of the specification.

In addition, new claims 20 and 21 which depend from claim 1 have been added.

Also, Applicant submits BLAST search results for the protein sequence of SEQ ID NO: 2 (glutaminase (gls)) and the nucleotide sequence of SEQ ID No: 3 (glutamine synthetase (gin)) attached herewith (Exhibit A and Exhibit B, respectfully) to support this argument.

The Examiner has rejected claim 1 and the claims dependent therefrom on the grounds that "means for reducing glutaminase activity" to the recited level is not adequately described by the specification and not fully enabled. Also, the Examiner stated that "a DNA which is able to hybridize with the DNA sequence of SEQ ID NO: 1 under stringent conditions of 1 X SSC, 0.1% SDS, at 60°C" is too broad and not fully enabled.

Claim 1 has been amended to recite that the homology level of the homologous DNA with SEQ ID NO: 1 is "95% or more". Also, based on the alignment of the protein sequences of glutaminase (gis), one of ordinary skill in the art would clearly recognize which regions are important for the enzymatic activities of the proteins, and would be able to reduce the activities of the proteins by introducing amino acid mutations at such regions, and hence the claims are fully and adequately described. Furthermore, one of ordinary skill in the art would be able to readily ascertain expression regulatory sequence of the recited glutaminase gene on the chromosome of a coryneform bacterium based on the sequence information for glutaminase genes, and would be able to mutate or disrupt

the expression regulatory sequence of the recited glutaminase gene. That is, one of ordinary skill in the art would be able to obtain a coryneform bacterium in which glutaminase activity is reduced to 0.1U/mg of cellular protein or less, by mutating or disrupting the recited glutaminase gene on the chromosome of a coryneform bacterium and/or by mutating or disrupting expression regulatory sequence of the recited glutaminase gene, based on the known sequence information for glutaminase genes, the teaching of the specification, and the knowledge and level of skill in this art. No undue experimentation is required to determine such information, and the invention is fully and adequately described, particularly in view of the knowledge in the prior art regarding the sequences.

Therefore, claim 1 is believed to be adequately described in the present specification and fully enabled.

The Examiner has rejected claims 5 and 16 and the claims dependent therefrom on the grounds that "means for increasing glutamine synthetase activity" is not adequately described by the specification and not fully enabled. Also, the Examiner stated that "a DNA which is able to hybridize with the DNA sequence of SEQ ID NO: 3 under stringent conditions of 1 x SSC, 0.1% SDS, at 60°C" is too broad and not fully enabled. However, claims 5 and 16 have been amended to recite that the homology level of the homologous DNA with SEQ ID NO: 3 is "95% or more". Also, as is evidenced by the alignment of the nucleotide sequences of glutamine synthetase gene (gin) from *B. flavum* (SEQ IDNO: 3), *C. glutamicum, C. efficiens,* and *Mycobacterium tuberculosis,* gln genes are highly conserved. Therefore, one of ordinary skill in the art would reasonably understand that a DNA sequence which has 95% or more homology to SEQ ID NO: 3 would naturally encode a protein having glutamine synthetase activity. Thus, one of ordinary skill in the art would reasonably be able to ascertain structures of the recited homologous gene which encodes a glutamine synthetase. Also, claims 5 and 16 have been amended to restrict means for increasing glutamine synthetase activity to "by increasing the copy number of glutamine synthetase gene" or "by replacing promoter region of glutamine synthetase gene with a stronger promoter". These two means are fully supported by the present specification (see page 17, lines 15—21) Therefore, one of ordinary skill in the art is believed to reasonably understand that by increasing the copy

number of the recited glutamine synthetase gene or by replacing promoter region of the recited glutamine synthetase gene with a stronger promoter, glutamine synthetase activity would be enhanced.

Therefore, claims 5 and 16 are believed to be adequately described in the present specification and fully enabled.

For at least the foregoing reasons, Applicant respectfully submits that the Claims fully comply with 35 U.S.C. § 112, first paragraph, and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 112.

Rejection under 35 U.S.C. § 103(a)

In the Office Action, beginning at page 9, Claims 1, 4-5, 7, 13-17, and 19 were rejected under 35 U.S.C. § 103(a), as reciting subject matters that allegedly are obvious, and therefore allegedly unpatentable, over the disclosure of Nakamura et al. in view of the disclosure of Pompejus et al. and further in view of Duran et al. Applicant respectfully requests reconsideration of this rejection.

Duran et al. teach that the glutaminase-deficient mutant strain of *Rhizobium etli* (LM16) demonstrated an increased intracellular glutamine level when the mutant strain was cultivated in the presence of glutamine as the carbon source (Table 1). However, this reference only teaches uptake of glutamine added to the medium by the bacterial cells, and does not show production of glutamine by the LM16 strain in the culture medium. It can be interpreted that the phenomenon that "intracellular glutamine levels are increased when glutamine is used as a carbon source" arises because glutamine which has been imported into the bacterial cells is not degraded in the absence of glutaminase. Furthermore, when succinate and glutamine were simultaneously added to the medium, intracellular glutamine levels decreased as compared to when glutamine was used as the sole carbon source. Such strain is not believed to have the ability to produce glutamine.

On the other hand, the bacterium as claimed in the present application is a coryneform bacterium which has L-glutamine-producing ability. As described in the specification of the present application (see page 6, lines 17-19), "L-glutamine-producing ability" means an ability of the bacterium to accumulate L-glutamine in a medium when

the bacterium is cultivated in the medium. Such bacterium having L-glutamine-producing ability is believed not to be disclosed or suggested by Duran et al.

Meanwhile, *Rhizobium* bacterium is known to have an enzyme which catalyzes the formation of 2 molecules of glutamic acid from 1 molecule of glutamine and 1 molecule of α-ketoglutarate (αKG) in the presence of NADPH. This enzyme is called GOGAT, and is also the case with coryneform bacterium. GOGAT does not function in the absence of αKG.

As is seen in Table 1 of Duran et al., glutamine accumulates in the cells when glutamine is added as the sole carbon source. This is probably because when glutamine is used as the sole carbon source, αKG is not produced in the cells and GOGAT does not work. On the other hand, simultaneous addition of succinate and glutamine lead to a marked decrease in intracellular glutamine levels as compared to when glutamine is the sole carbon source. This is probably because GOGAT works to degrade glutamine in the presence of succinate. The reason why GOGAT works to degrade glutamine in the presence of succinate is that when succinate is added, αKG is produced via the TCA cycle. Also, because pyruvate is formed from succinate, and then acetyl CoA is formed from pyruvate, the TCA cycle can use the produced acetyl CoA.

It is well known that glutamine fermentation is typically performed in the presence of glucose. During such fermentation, αKG accumulates in the fermentation broth. Therefore, a sufficient amount of αKG is present in the cells when the cells are cultivated in the presence of glucose. Also, producing αKG from glucose (C6→C5) is more efficient than producing αKG from succinate (C4→C5), because the former does not need an extra metabolic pathway. Therefore, GOGAT would be expected to have a greater contribution to the degradation of glutamine when glucose is added to the medium, as compared to when succinate is added to the medium.

In view of the above, Duran et al. clearly do not teach or suggest an increased glutamine level in a glutaminase-deficient bacterium when glucose is present in the medium. In fact, the teaching of Duran et al. is actually to the opposite, that in the presence of glucose, glutamine levels in such bacterium would be decreased.

Nakamura et al. teach glutamine fermentation by a coryneform bacterium, which is typically performed in the presence of glucose in the medium. However, there is no

teaching in Nakamura et al. of disabling the glutaminase gene. Pompejus et al. teach the genes encoding the glutaminase and glutamine synthetase genes from *C. glutamicum*, which applicants do not dispute. However, there is no reason, for the reasons stated above, that one of ordinary skill in the art would be motivated or have any reason to combine the teachings of these three references. Most notably, this is because, one of ordinary skill in the art would have reasonably concluded that the glutamine level in bacterial cells would decrease, based on the teachings of Nakamura et al. combined with the teaching of Duran et al. and Pompejus et al., which is opposite to the effect of the claimed invention.

Thus, there is no reason or motivation to combine Nakamura et al., Duran et al., and/or Pompejus et al. for the purpose of breeding a glutamine-producing bacterium. Furthermore, through no combination of the cited references is it obvious for one of ordinary skill in the art to modify a coryneform bacterium to reduce intracellular glutaminase activity with the expectation of obtaining a bacterium with enhanced L-glutamine producing ability.

For at least the foregoing reasons, Applicant respectfully submits that the subject matters of the Claims, each taken as a whole, would not have been obvious to one of ordinary skill in the art at the time of Applicant's invention, are therefore not unpatentable under 35 U.S.C. § 103(a), and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 103(a).

Conclusion

For at least the foregoing reasons, Applicant respectfully submits that the present patent application is in condition for allowance. An early indication of the allowability of the present patent application is therefore respectfully solicited.

If Examiner Ramirez believes that a telephone conference with the undersigned would expedite passage of the present patent application to issue, she is invited to call on the number below.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to our Deposit Account 50-2821.

Respectfully submitted,

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